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BIODISTRIBUTION OF MILD ANALGESICS

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1 Macro-autoradiographic methods were used to assess the biodistribution of [^{3}H]-, or [^{14}C]-acidic (aspirin, indomethacin, phenylbutazone) and non-acidic (antipyrine, aminopyrine, paracetamol) mild analgesics in rats with carrageenan-induced inflammation.

2 At anti-inflammatory doses all the acidic drugs (and/or their metabolites) were found to reach high concentrations in the stomach wall, liver, blood and bone marrow, kidney cortex and the inflamed tissue, that is, the tissues in which these drugs exert their therapeutic or side-effects.

3 In contrast, at analgesic doses, the non-acidic mild analgesics (and/or their metabolites) are equally distributed throughout the body with the exception of the gastro-intestinal lumen and the liver. This distribution pattern correlates well with the lack of acute side-effects and anti-inflammatory action of these drugs at therapeutic doses.

Introduction

DESPITE much research during the past decade, little is known about the molecular basis of action of the so-called mild analgesics. These drugs, in contrast to the opioids, have not been found to mimic physiological mediator(s) of pain. Neither have they been observed to block or activate a specific, molecularly defined receptor. Instead, these drugs, which have to be administered in gram quantities in man, seem to interfere with a variety of cellular and molecular functions, and thus can be assumed to have multifactorial actions (Brune, 1974; Vane, 1980). If this assumption is correct, any effect of mild analgesics in a particular compartment should be directly related to the concentration of the drug therein because specific receptors do not exist. Nevertheless, these compounds may exert a specific pattern of effects and side-effects simply by distributing unequally through the body. A corollary is that these drugs should accumulate in all the compartments in which they show pharmacological effects or side-effects.

In experiments reported previously (Graf, Glatt & Brune, 1975; Brune, Glatt & Graf, 1976), we have shown that radioactively labelled acidic anti-inflammatory drugs administered in sub anti-inflammatory doses to young (30 g) rats reached high concentrations in tissues in which they exert effects or side-effects. We have now extended these observations to large (120 g) rats, using labelled acidic and non-acidic analgesics in high, that is, anti-inflammatory or analgesic doses, respectively.

Methods

Male Sprague-Dawley rats (100–130 g body weight, obtained from Tierfarm, Tuttlingen, FRG) were subjected to an acute (local) inflammatory reaction by inoculation with 0.05 ml 2% w/v carrageenan in sterile saline into the left rear paws. An injection of 0.05 ml sterile saline into the right rear paw served as control. The animals were then dosed with 1 ml aqueous fine suspensions, or solutions of the radioactively labelled drugs (details below) prepared immediately beforehand. Blood samples (50–200 μl) were then collected from the tail veins at 10 and 60 min after dosing and finally on termination at 180 min from the carotid arteries or veins (under ether anaesthesia). These blood samples were assayed for radioactive content following total combustion.

After collection of the final blood sample the rats were bled as completely as possible while anaesthetized, and the shaven carcasses were rapidly frozen in hexane-dry ice mixture. The legs and tails were removed, the carcasses embedded in sodium carboxymethyl cellulose for subsequent sectioning on an LKB 2250 PMV Cryo-Microtome (PMV 450 MP). The rear paws were likewise embedded and sectioned separately. Approximately 100 μm thick sections were obtained and subsequently freeze-dried. Whole body autoradiographs of [^{14}C]-labelled rats were then prepared by exposing the freeze-dried sections to direct contact with X-ray film.

Animals dosed with [^{3}H]-labelled drugs were exposed to Ultrofilm-^{3}H® (LKB, Bromma, Sweden) at —25°C. These were developed in batches at 1–6

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weeks following exposure. Freeze-dried sections and saline homogenates of the frozen sections remaining after selectioning of the paws were digested in Protosol tissue solubilizer (New England Nuclear, Boston, Massachusetts, USA), and the radioactivity determined after addition of Instagel (Packard) scintillation mixture. The radioactive content in the tissue homogenates and blood was determined in a Packard Tri-carb Scintillation Spectrometer (Packard Instrument Co., Boston, Massachusetts, USA) and the data corrected for quenching by the channels ratio method. Losses of radioactively labelled drug on dosing syringes, test tubes, needles, and so on, were determined by washing and counting the residues. In most cases these accounted for 5% of the dose administered and appropriate corrections were derived in calculation of the fraction of the dose in blood (see later).

Radioactively labelled drugs

[^{3}H]-acetylsalicylic acid synthesized (Rainsford, Schweitzer & Brune, unpublished), N-methyl-[^{14}C]-antipyrine, aminopyrine ([^{14}C]-dimethylamine) both from New England Nuclear, Boston, Massachusetts, USA) and ^{14}C-phenylbutazone (gift of Ciba-Geigy Ltd, Basel, Switzerland) were mixed with non-radioactively labelled drugs to give doses of 100 μCi in 100 mg drug/kg body weight. Paracetamol (p-[^{3}H]-hydroxyacetanilide; New England Nuclear) was mixed with non-radioactive drug to give a dose of 2.5 mCi in 100 mg drug/kg body weight. 2-[^{14}C]-indomethacin (gift from Merck Institute for Therapeutic Research, Rahway, New Jersey, USA) was added to non-labelled drug to achieve doses of 200 μCi in 10 mg drug/kg body weight. The purity of all radioactivity labelled drugs was determined before use by thin layer chromatography and radio-chromatogram scanning (Rainsford, Schweitzer & Brune, in preparation).

Results

Distribution of acidic, anti-inflammatory analgesics

The results of distribution studies using acetylsalicylic acid, phenylbutazone and indomethacin are shown in Figures 1 and 2 and Table 1. It is evident that rapid absorption occurs after oral administration. Appreciable concentrations are present in blood at 10 min (Figure 1). Concentrations appear to plateau at about 1 h (acetylsalicylic acid), 3 h (indomethacin) or even later with phenylbutazone. This observation corresponds with the autoradiographic findings. At 3 h activity from the acetyl moiety of acetylsalicylic acid is present especially in the stomach wall (Figure 2*a*) whereas substantial amounts of phenylbutazone (Figure 2*b*) and indomethacin (Figure 2*c*) are present in the stomach lumen as well. Within the body all the three acidic drugs show a similar distribution pattern in reaching particularly low concentrations in the CNS but with high concentrations in the stomach wall, blood, liver, renal cortex and inflamed tissue. It is noteworthy that radioactivity derived from the acetyl group of labelled aspirin is also clearly concentrated in

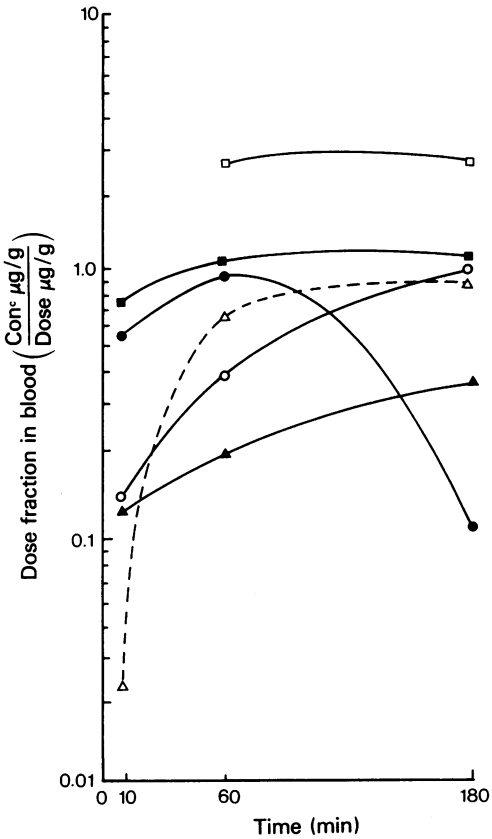

Figure 1 Fraction of dose in whole blood at different times after oral administration of analgesic drugs. The [^{3}H]- or [^{14}C]-labelled drugs were administered at zero time and blood sampled at the times given. Total radioactivity (corrected for quenching and expressed as d.m.p./mg) was used to calculate the amount of drug present in blood; with no corrections for conversion of parent drug to metabolites being applied. Fraction of dose in blood was then calculated, which refers to the calculated concentration of drug in blood divided by dose given. □, [^{3}H]-acetylsalicylic acid; ■, indomethacin; ○, phenylbutazone; △, antipyrine; ▲, aminopyrine; ●, paracetamol.

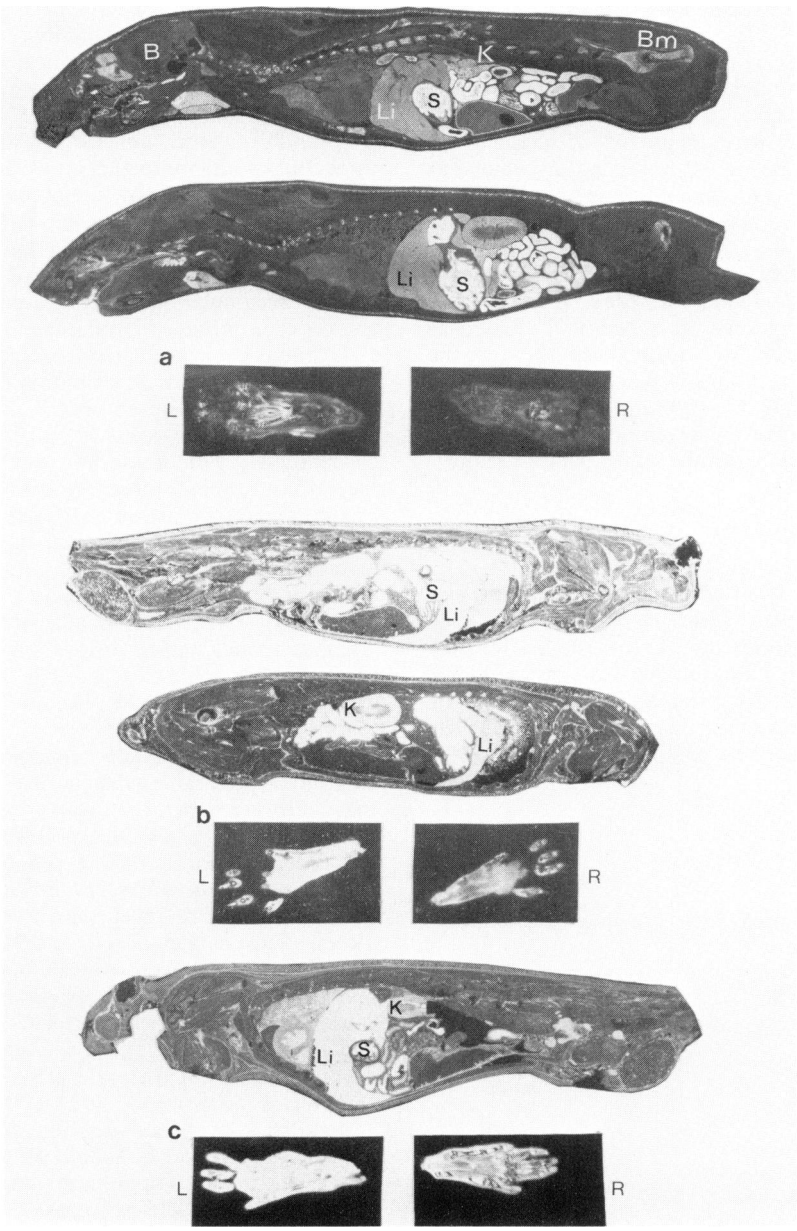

Figure 2 Autoradiographs from rats treated with [^{3}H]-acetylsalicylic acid (*a*), [^{14}C]-phenylbutazone (*b*), and 2-[^{14}C]-indomethacin (*c*). Drugs were given orally in anti-inflammatory doses (for details see Methods sections). At the same time inflammation was elicited in the left (L) hind paw. Three hours later the animals were exsanguinated, deep-frozen and cut in thin (100 μm) slices, which were lyophilized and exposed on X-ray film for 4 weeks. B, Brain; Bm, bone marrow; Li, liver; K, kidney; S, stomach. Lighter regions correspond to intense areas of radioactivity.

the bone marrow. Table 1 shows that at the high doses administered the difference in drug concentration (that is, radioactivity) is most prominent with indomethacin; this being almost three times higher in concentration in the inflamed compared with the control paw.

Distribution of non-acidic 'mild' analgesics

Similar experiments using the non-acidic 'mild' analgesics paracetamol, antipyrine and aminopyrine gave different results to those observed with the acidic drugs. The early phase of absorption was delayed with antipyrine. However, paracetamol was more rapidly absorbed than antipyrine but the blood concentration of paracetamol began to decrease rapidly after about 1 hour. Within the organs of body the radioactivity is apparently distributed quite homogeneously, including the brain (Figure 3*a*, *b* and *c*). Also, there is no substantial difference in the concentration of radioactivity between the inflamed and non-inflamed paw with these non-acidic analgesics (Figure 3*a*, *b* and *c*; Table 1). High concentrations of these drugs are only present in the liver, the gastric and intestinal lumen, and the kidney.

Discussion

The results obtained suffer from the usual deficiencies of autoradiographic studies; that is, we have monitored the biodistribution of the radioactive isotope but this distribution of radioactivity represents both active drug and metabolites. An analysis of the chemical nature of the labelled molecules in the different compartments of interest is in preparation.

Table 1 Relative concentrations of radioactivity in the inflamed paws

Type of drug	*Compound*	p*Ka*	*Dose (mg/kg)*	*Percentage increase in the inflamed paw*‡
Anti-inflammatory*	Aspirin	3.5	100	45
Acidic	Phenylbutazone	4.8	100	81
Analgesics	Indomethacin	5.2	10	183
Non-acidic†	Paracetamol §	9.5	100	12
Mild	Antipyrine ¶	1.4	100	21
Analgesics	Aminopyrine	5.0	100	28

Doses chosen are those required to achieve anti-inflammatory*, or analgesic† activity, respectively.

‡ Calculated from

$$\frac{\text{d.m.p./mg drug weight in inflamed paw}}{\text{d.m.p./mg dry weight in control paw}} \times 100$$

Values are the average of two paw sets.

§ Very weak acid, under physical conditions not ionized.

¶Very weak base, under physical conditions not ionized.

ionized.

We have confirmed our previous observations concerning the specific biodistribution of acidic, anti-inflammatory analgesics using near adult rats (120 g) at high, clearly anti-inflammatory doses (see also the data of Atkinson & Leach, 1976). Even at these doses considerably higher concentrations (for example, three times with indomethacin) of labelled drug were found in the inflamed paw compared with the control. In contrast, the non-acidic drugs (which do not have anti-inflammatory action at the doses given) did not accumulate so much as the acidic drugs. Hence, the specific accumulation of acidic, anti-inflammatory analgesics in inflamed tissue (Cummings & Nordby, 1966) and other acidic compartments of the body.

The relevance of biodistribution in understanding the effects and side-effects of the acidic, anti-inflammatory analgesics is supported by these observations. The non-acidic analgesics with the exception of aminopyrine (see below) are devoid of anti-inflammatory action in therapeutic doses and correspondingly do not accumulate in the inflamed tissue. Aminopyrine has some weak anti-inflammatory effects (Beaver, 1965; 1966) and this drug shows some, albeit small, accumulation in the inflamed tissue. As this drug is a moderately strong base (*p*Ka 5) it would be expected to accumulate in the slightly acidic extracellular, but not the intracellular, space of inflamed tissue.

However, extracellular accumulation of these drugs seems to be irrelevant to their actions in comparison to intracellular accumulation of acidic anti-inflammatory/analgesic drugs (Brune & Graf, 1978). This interpretation is further supported by the fact that all non-acidic drugs accumulate in appreciable quantities in the stomach lumen, but do not cause ulcers. The acidic drugs, on the other hand, accumulate in the glandular mucosa of the stomach and the kidney cortex. Correspondingly, these drugs cause ulcer formation (Rainsford, 1975), electrolyte and water retention, or even acute pathological changes in the kidney (Morales & Steyn, 1971; Arnold, Collins & Starmer, 1974). Moreover, the accumulation of radioactivity from [^{3}H]-acetyl groups of acetylsalicylic acid in the bone marow may be taken as an indication of acetylation of proteins as, for example, the cyclo-oxygenase of megakaryocytes in the bone marrow. This could explain the long-lasting (5 d) inhibition of platelet aggregation by acetylsalicylic acid.

Finally, it should be pointed out that with the exception of the liver, relatively high concentrations of radioactivity are evident following dosage with [^{14}C]-aminopyrine in the bone marrow, the skin and the oral mucosa. It is tempting to suggest that the accumulation of drug in these tissues may be related to the occurrence of antibodies against components of these tissues and consequently side-effects — namely agranulocytosis and skin and mucosal

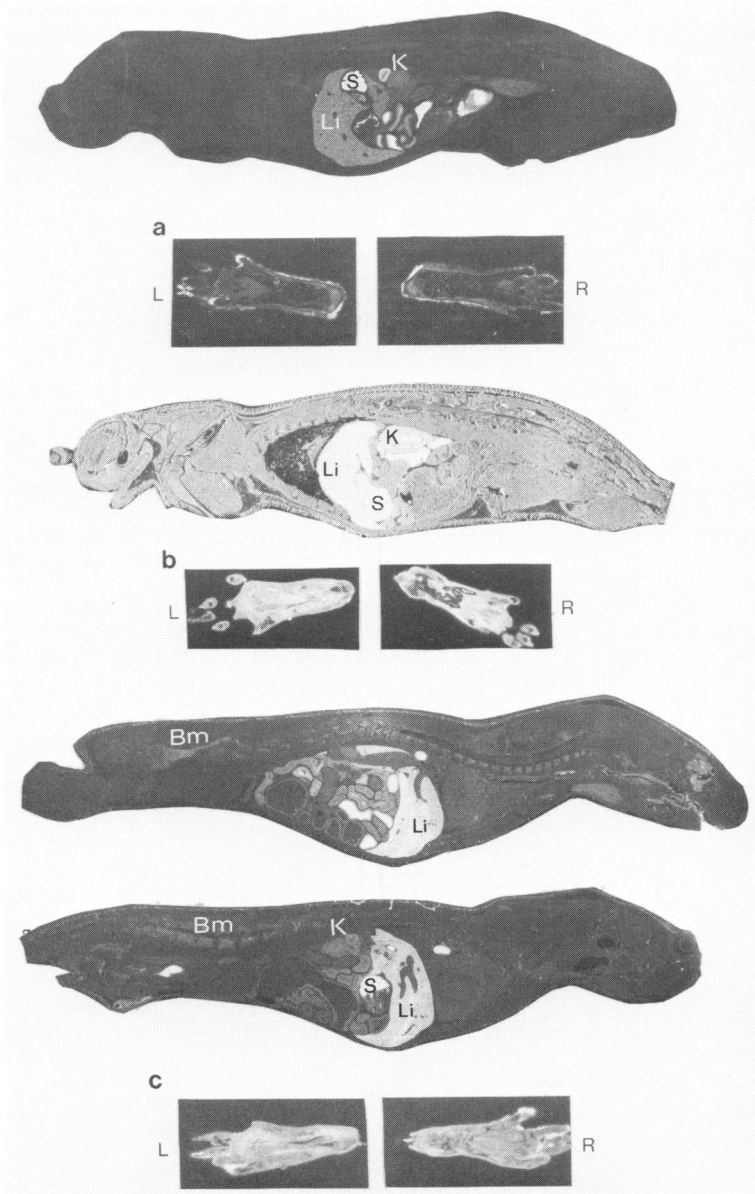

Figure 3 Autoradiographs from rats treated with [^{3}H]-paracetamol (*a*), [^{14}C]-N-methyl-antipyrine (*b*) and [^{14}C]-dimethylamine (aminopyrine) (*c*). Drugs were given orally in analgesic doses (for details see Methods section). At the same time an inflammatory reaction was elicited in the left (L) hind paw. Three hours later, the animals were exsanguinated, deep-frozen and cut in thin (100 μm) slices, which were lyophilized and exposed on X-ray film for 4 weeks (*a, b*) or 6 weeks (*c*). Abbreviations as in Figure 2. Lighter regions correspond to intense areas of radioactivity.

eruptions (Beaver, 1965; 1966). Paracetamol, on the other hand, leaves the body of the rat quite rapidly but substantial amounts of radioactivity are retained in the liver.

Our autoradiographs are consistent in this respect with the proposed mechanisms of paracetamol hepatoxicity discussed in other contributions to this workshop.

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